526 Rec'd PCT/PTO 10 JUL 2001

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 4121-126
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U S APPLICATION NO (If known, see 37 CFR 1 5) 09/889182
INTERNATIONAL APPLICATION NO. PCT/DE00/00079	INTERNATIONAL FILING DATE 11 January 2000	PRIORITY DATE CLAIMED 11 January 1999

TITLE OF INVENTION

SELECTION OF MONOCLONAL ANTIBODIES

APPLICANT(S) FOR DO/EO/US

Frank Breitling, Annemarie Poustka and Gerard Moldenhauer

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U S C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).***(Unsigned)**
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☒ A small entity statement.
16. ☐ Other items or information: EPO Search Report in German

NOTE: This application is being filed with an unsigned Oath or Declaration under the provisions of 37 CFR § 1.53 in order that applicants may secure a filing date of July 10, 2001. Upon receipt of a "Notice to File Missing Parts - Filing Date Granted," a executed Declaration and Power of Attorney will be forwarded. The undersigned agent affirmatively states that she has been duly authorized and appointed to file this application on behalf of the applicants and applicants' assignees, and that the Declaration and Power of Attorney to be filed hereafter will confirm the undersigned agent's authorization and appointment. Applicants are considered a small entity and assignee Deutsches Krebsforschungszentrum is also considered a small entity within the meaning of 37 CFR § 1.9.

09/889182

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17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO ....$860 International preliminary examination fee paid to USPTO (37 CFR 1.482) ....$0.00 No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ....$0.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ....$1000.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $0.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20-20 =	0	X **$18.00**	$	
Independent Claims	2- 3 =	0	X **$80.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1 27, 1 28).				$ 430.00	
SUBTOTAL =				$ 430.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEE ENCLOSED =				$ 430.00	
				Amount to be: refunded	$
				Charged	$

a. ☒ A check in the amount of $430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _______ in the amount of $_______ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

Marianne Fuierer

MARIANNE FUIERER
Registration No. 39,983

SEND ALL CORRESPONDENCE TO:

Steven J. Hultquist
Intellectual Property/Technology Law
P. O. Box 14329
Research Triangle Park, NC 27709

09/889182

JC18 Rec'd PCT/PTO 10 JUL 2001

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO ....$860.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) ....$0.00 No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ....$0.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ....$1000.00 International preliminary examination fee paid to USPTO (37 CFR 1 482) and all claims satisfied provisions of PCT Article 33(2)-(4). $0 00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20-20 =	0	X **$18.00**	$	
Independent Claims	2- 3 =	0	X **$80.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 430.00	
SUBTOTAL =				$ 430.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1 21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEE ENCLOSED =				$ 430.00	
				Amount to be: refunded	$
				Charged	$

a. ☒ A check in the amount of $430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _______ in the amount of $_______ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

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JC14 Rec'd PCT/PTO 03 JAN 2002

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4121-126
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

23448
PATENT & TRADEMARK OFFICE

In re Application of:	**BREITLING, et al.**
Application No.:	**09/889,182**
International Application No.:	**PCT/DE00/00079**
Priority Date Claimed:	**11 January 2000 and 11 January 1999 (German Appl. No. 199 00 635.0)**
Title:	**SELECTION OF MONOCLONAL ANTIBODIES**

FIRST CLASS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Washington, DC 20231, and First Class Mailed under the provisions of 37 CFR 1.8.

Lee Ann Brown

Lee Ann Brown

November 14, 2001
Date of Mailing

09889182.011002

SUPPLEMENTAL PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified national phase patent application, please amend the application, as follows:

In the Specification

Please insert on page 1 between the title of the application and the first paragraph the following new paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is filed under the provisions of 35 U. S.C. §371 and claims the priority of International Patent Application No. PCT/DE00/00079 filed January 11, 2000, and which in turn claims priority of German Patent Application No. 199 00 635.0 filed January 11, 1999.

REMARKS

This claim to priority is being filed before the above-identified application meets all requirements under 35 U.S.C. §371(b).

Respectfully submitted,

Marianne Fuierer
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Attorney for Applicants

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09/889182
JC18 Rec'd PCT/PTO 10 JUL 2001

4121-126
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**BREITLING, et al.**
Application No.:	New U.S. National Stage Application of PCT International Application No. PCT/DE00/00079
International Filing Date:	11 January 2000
Priority Date Claimed:	11 January 1999 (German Appl. No. 199 00 635.0)
U.S. National Phase Filing Date:	Date of mailing identified below
Title:	**SELECTION OF MONOCLONAL ANTIBODIES**

EXPRESS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Box Patent Application, Washington, DC 20231, and Express Mailed under the provisions of 37 CFR 1 10

Blake Crouch
Name of Person Mailing This Document

Blake C
Signature

July 10, 2001
Date

EL666414295US
Express Mail Label Number

PRELIMINARY AMENDMENT

Commissioner for Patents
BOX PATENT APPLICATION
Washington, D.C. 20231

Sir:

09889182.071001

Prior to examination of the above-identified new national phase patent application, please amend the application, as follows:

In the Specification

On the bottom of page 4 and top of page 5, please replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID. NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2)of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431) and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

In the Claims

Please amend claims 1-20 to read as follows:

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to

antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.

2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.

3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.

4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.

5. The method according to claim 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 and CD64.

6. The method according to claim 2, wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and LG.

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a signal peptide selected from the group consisting of a signal peptide of a mouse Ig cappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein selected from the group consisting of protein A, G, L, and LG; and a transmembrane domain selected from the group consisting of PDGFR and CD52.

8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6.

9. The method according to claim 1, wherein the hybridoma cells (over)express Rag1 and/or Rag2.

10. The method according to claim 1, wherein the antigens originate from an antigen library.

11. The method according to claim 1, wherein the antigens are bound to a carrier.

12. The method according to claim 11, wherein the carrier comprises magnetobeads.

13. The method according to claim 7, wherein the antigens comprise a fluorescence or biotin labeling.

14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.

15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L and LG, and a transmembrane domain selected from the group consisting of PDGFR and CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.

17. DNA coding for the antibody binding protein according to claim 16, comprising:
    (a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431

[of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or

(b) a DNA related to the DNA of (a) via the degenerated code.

18. An expression vector, coding for the DNA according to claim 17.

19. Cells containing the expression vector according to claim 18.

20. An antibody directed against the antibody binding protein according to claim 16.

REMARKS

A marked-up version of amended paragraph in the specification and amended claims 1-20 are included herewith in Appendix A.

It is requested that the examination and prosecution of this application proceed on the basis of the English translation of the PCT International application included herewith and these amended claims 1-20.

Respectfully submitted,

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APPENDIX A

In the Specification

On the bottom of page 4 and top of page 5, replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID. NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2)of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431)and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

In the Claims

5. The method according to claim [3 or] 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 [or] and CD64.

6. The method according to claim 2 [any of claims 2 to 5], wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and [or] LG.

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a [the] signal peptide selected from the group consisting of a signal peptide of a mouse Ig cappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein[s] selected from the group consisting of protein A, G, L, and [or] LG; and [the] a transmembrane domain selected from the group consisting of PDGFR [or] and CD52.

8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6. [that of figure 1, figure 2 or figure 3.]

9. The method according to claim 1 [any of claims 1 to 8], wherein the hybridoma cells (over)express Rag1 and/or Rag2.

10. The method according to claim 1 [any of claims 1 to 9], wherein the antigens originate from an antigen library.

11. The method according to claim 1 [any of claims 1 to 10], wherein the antigens are bound to a carrier.

13. The method according to claim 7, [any of claims 1 to 10], wherein the antigens comprise a fluorescence or biotin labeling.

15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain [or] and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L [or] and LG and a [the] transmembrane domain selected from the group consisting of PDGFR [or] and CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an [the] amino acid sequence selected from the group consisting of [figure 1, figure 2 or figure 3] SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.

17. DNA coding for the antibody binding protein according to claim 16, comprising:
    (a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431 [of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or
    (b) a DNA related to the DNA of (a) via the degenerated code.

Selection of Monoclonal Antibodies

The present invention relates to a method of selecting monoclonal antibodies and to means which can be used therefor.

The production of monoclonal antibodies is based on a method developed by Kohler and Milstein. According to this method B lymphocytes are fused with myeloma cells so as to obtain antibody-producing hybridoma cells. Such a method comprises major drawbacks. In particular, it is time-consuming and expensive to select antibodies, since this calls for separate culturing of hybridoma cells. Due to the latter only a limited number of hybridoma cells is detected and thus not all of the antibodies can be selected, this being a drawback in particular when antibodies with maximum affinity for an antigen shall be selected.

It is thus the object of the present invention to provide a product by which monoclonal antibodies can be produced, the above drawbacks being avoided.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on Applicant's insights that monoclonal antibodies on the cell surface of hybridoma cells can be presented by means of an antibody binding protein. He realized that monoclonal antibodies can be selected by this without hybridoma cells having to be cultured separately. He also realized that monoclonal antibodies can be selected

with respect to a determined and many (un)determined antigens of an antigen library. Furthermore, he found that monoclonal antibodies can also be selected with respect to their affinity intensity for certain antigens.

According to the invention Applicant's insights are used to provide a method of selecting monoclonal antibodies. Such a method comprises fusing B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and binding of the antibodies to antigens.

The expression "B lymphocytes" comprises B lymphocytes of any kind and origin. They may also concern precursors of B lymphocytes. The B lymphocytes may originate from animals, such as mice, rats, rabbits, etc., or humans. The B lymphocytes may also originate from a healthy or diseased organism. It is favorable for them to originate from an immunized organism. It is particularly favorable for the B lymphocytes to code for human antibodies or portions thereof. If B lymphocytes from animals are concerned, this can be achieved when the animals are transgenic for the human antibodies or portions thereof. Such animals can be produced by common methods, it being an obvious thing to introduce the genes for the human antibodies or the portions thereof into embryonal stem cells from which the animals are then generated. B lymphocytes and their precursors may be provided by common methods.

The expression "myeloma cells" comprises myeloma cells of any kind and origin. They may also concern precursors of myeloma cells. Furthermore, the myeloma cells may originate from animals, such as mice, rats, rabbits, etc., or humans.

Preferred myeloma cells are descendents from the mouse strains P3K, P3-X63.Ag8, X63.Ag8.653, NSO/1, Sp2/O-Ag14 and FO, the rat strains Y3-Ag1.2.3, YB2/0 and IR9834, and the human strains U266, SK007 and Karpas 707. Myeloma cells and their precursors can be provided by common methods.

The expression "antibody-producing hybridoma cells" comprises cells which form by fusion of B lymphocytes and myeloma cells and produce antibodies. Corresponding reference is made to the statements on B lymphocytes and myeloma cells. Hybridoma cells may include animal and/or human nucleic acids and/or proteins. Hybridoma cells can be cultured by common methods. It may also be favorable for the hybridoma cells to (over)express recombinases, e.g. Rag1 or Rag2, and/or mutases. This can be achieved by transfection of the hybridoma cells with corresponding expression vectors. The person skilled in the art knows such expression vectors.

The term "fusion of B lymphocytes with myeloma cells" concerns any method by means of which these cells may be fused. A method is favorable in which the cells are fused via polyethylene glycol. Reference is made to the examples.

The term "binding of the antibodies to antigens" concerns any method by which the antibodies expressed on the cell surface of the hybridoma cells can bind to antigens. The antigens can be bound to carriers, e.g. magnetobeads. They can also be labeled, e.g. fluorescence-labeled. For example FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin offer themselves as fluorescence markers. The antigens may also be coupled to biotin. Bound antigens may be detected by common methods, e.g. FACS analysis whereby the corresponding

antibodies are also detected. Reference is made to the examples.

The expression "antibody binding protein" comprises any protein which may bind an antibody and present it on the cell surface of hybridoma cells. In particular, the protein may have a signal peptide, an antibody-binding site independent of the specificity of the antibody and a membrane anchor. Examples of such a protein are natural Fc binding proteins, such as CD16, CD32 and CD64. The protein may comprise a combination of a signal peptide, an antibody binding site and a membrane anchor, which does not occur in nature. Such a combination may comprise portions of natural Fc binding proteins. Furthermore, as a signal peptide it may have one of a mouse Ig kappa chain or a mouse MHC-class I k(k) molecule, as a membrane anchor it may include a transmembrane domain of PDGRF or CD52 and as an antibody binding site it may comprise an antigen binding domain of a bacterial protein, such as protein A, protein G, protein L or protein LG. It may be favorable for the combination to comprise several signal peptides, antibody binding sites and/or membrane anchors. It may be particularly favorable for the antibody binding protein, in particular the antibody binding domain of the bacterial proteins, to have codons which are optimized for expression in mammalian cells. A person skilled in the art knows which codons are concerned here.

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are given between

nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA and amino acid sequences of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

An antibody binding protein of figures 1, 2 or 3 may have an amino acid sequence which differs from the amino acid sequence in figure 1, 2 or 3 by one or more amino acids. The differences may lie in additions, deletions, substitutions and/or inversions of individual amino acids. However, the DNA of this antibody binding protein hybridizes with the DNA indicated in figure 1, 2 or 3. The term "hybridizing" refers to hybridization under common conditions, in particular at 20°C below the melting point of the DNA. Furthermore, the antibody binding protein having the modified amino acid sequence comprises whole or partial functions which can be compared with those of the antibody binding protein of figure 1, 2 or 3.

Another subject matter of the present invention relates to a nucleic acid which codes for an above antibody binding protein. The nucleic acid may be an RNA or a DNA. Preferred is a DNA which comprises the following:

(a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or

(b) a DNA related to the DNA from (a) by the degenerated genetic code.

The term "a DNA differing by one or more base pairs" comprises any DNA coding for an antibody binding protein of figure 1, 2 or 3, which hybridizes with the DNA of figure 1, 2 or 3. The differences may lie in additions, deletions, substitutions and/or inversions of individual base pairs. As to the term "hybridizing" reference is made to the above explanations.

A DNA according to the invention may be present as such or in combination with any other DNA. In particular, a DNA according to the invention, which codes for an antibody binding protein, may be present in an expression vector. The person skilled in the art is familiar with examples thereof. In the case of an expression vector for *E. coli* these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, pCDM8 and pCEV4 have to be indicated for the expression in animal cells. The bacculovirus expression vector pAcSGHisNT-A is particularly suitable for the expression in insect cells.

The person skilled in the art knows how to insert the DNA according to the invention in an expression vector. He also knows that this DNA can be inserted in combination with a DNA coding for another protein or peptide, so that the DNA according to the invention can be expressed in the form of a fusion protein.

Preferred expression vectors which contain a DNA according to the invention are shown in figures 1 to 3. The expression vectors pSEX11L4, pSEX11G2* and pSEX15G2 are concerned. They were deposited with the DSMZ (*Deutsche Sammlung für Mikroorganismen und Zellkulturen* [German-type collection of microorganisms and cell cultures]) on December 14, 1998. In particular, pSEX11L4 was deposited under DSM 12580, pSex11G2* was deposited under DSM 12581 and pSEX15G2 was deposited under DSM 12582.

The person skilled in the art is familiar with suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the E. *coli* strains XL-1 Blue, Top 10 F, HB101, DH5alpha, x1776, JM101, JM 109, BL21 and SG 13009, the yeast strain *Saccharomyces cerevisiae* and *Pichia pastoris*, the animal cells L, NIH 3T3, FM3A, CHO, COS, Vero, HeLa, myeloma and hybridoma cells as well as the insect cells sf9.

The person skilled in the art also knows conditions of culturing transformed or transfected cells. He is also familiar with methods of isolating and purifying the protein or fusion protein expressed by the cDNA according to the invention.

Another subject matter of the present invention relates to an antibody directed against an above protein or fusion protein. Such an antibody may be prepared by common methods. It may be polyclonal or monoclonal. For its preparation it is favorable to immunize animals - in particular rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above (fusion) protein or with fragments thereof. Further "boosters" of the animals can be effected

with the same (fusion) protein or with fragments thereof. The polyclonal antibody may then be obtained from the animal serum or egg. For the preparation of the monoclonal antibody, animal spleen cells are fused with myeloma cells.

Another subject matter of the present invention is a kit. Such a kit comprises one or more of the following components:

(a) a DNA according to the invention,
(b) a cell expressing a DNA according to the invention,
(c) an antibody binding protein according to the invention,
(d) an antibody according to the invention, and
(e) common auxiliary substances such as carriers, buffers, solvents, controls, markers, detection reagents for components (a) - (d).

One or more representatives of the individual components may be present. As to the individual terms reference is made to the above statements. They apply here analogously.

The present invention distinguishes itself in that antibodies produced by hybridoma cells are presented on the cell surface of the hybridoma cells. This is done via an antibody binding protein. Such a protein may be introduced into the hybridoma cells via the myeloma cells used for the production of the hybridoma cells. The antibody binding protein may also be introduced into the hybridoma cells via an expression vector coding for it.

By means of the present invention it is possible to select antibodies. This can be done without much expenditure, since hybridoma cells do not have to be cultured separately. Complex mixtures of hybridoma cells can rather be used

directly for selecting antibodies. Antibodies can also be selected for their affinity intensity for certain antigens. The present invention is also suitable for selecting antibodies of hybridoma cell libraries, not only with respect to a determined antigen but also as regards many (un)determined antigens of antigen libraries.

Thus, the present invention provides products by which it is possible to avoid *inter alia* major problems as regards time and cost which have occurred in the selection of monoclonal antibodies thus far.

Brief description of the drawings:

Fig. 1 shows the expression vector pSEX11L4 according to the invention (figure 1A) which codes for an antibody binding protein (figure 1(B)). Reference is made to the above explanations.

Fig. 3 shows the expression vector pSEX11G2* according to the invention (figure 2(A)), which codes for an antibody binding protein (figure 2(B)). Reference is made to the above explanations.

Figure 3 shows the expression vector pSEX15G2 according to the invention (figure 3(A)), which codes for an antibody binding protein (figure 3(B)). Reference is made to the above explanations.

The present invention is explained by the below examples.

Example 1: **Preparation of myeloma cells which express an antibody binding protein on their cell surface**

(A) Transient expression

Cells of the myeloma cell line X63-Ag8.653 are used. These cells (10^7) are transfected with 20-40 µg of the expression vector SEX11G2* according to the invention (cf. figure 2). Electroporation is carried out as transfection technique, which comprises two pulses of 2 ms at 500 V. The cells are incubated for 48 h at 37°C and 5-7.5 % Co_2 in RPMI medium which contains 10 % FCS. Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide plus 25 µg/ml goat anti-calf antibody (FITC-labeled; GAB-FITC, Dianova company). Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1 µg/ml propidium iodide and subjected to FACS analysis following excitation with blue light.

It shows that the transfected myeloma cells have a green fluorescence which is due to the transient expression of an antibody binding protein on the cell surface of the myeloma cells.

(B) Stable expression

The myeloma cells obtained under (A) are subjected to G418 selection for 14-24 days before they are incubated using GAB-FITC and subjected to FACS analysis as described under (A). Myeloma cells which have a strong green fluorescence are subject to further G418 selection rounds or runs.

The myeloma cell line X63-Ag8.653.3 is obtained which stably expresses an antibody binding protein on its cell surface.

Example 2: Production of hybridoma cells which express on their cell surface antibodies by means of an antibody binding protein

(A)

10 Balb/c mice are immunized subcutaneously in each case with 100 µg killed *Helicobacter pylori* bacteria in complete Freund's adjuvant, which contains killed *Mycobacter tuberculosis* bacteria. After 4 or 7 weeks, an intraperitoneal booster injection with 100 µg killed *Helicobacter pylori/Mycobacter tuberculosis* bacteria is given. 100 µl blood serum are withdrawn from the mice before each immunization and after the last immunization, and the antigen-specific immune response of the mouse is tested in a Western blot. A degradation of bacterial whole protein of *Helicobacter pylori* and/or *Mycobacter tuberculosis* is used as antigen. The detection of bound mouse antibodies is made by an peroxidase-conjugated goat anti-mouse antibody (Dianova company). The spleen of mice having a marked antigen-specific immune response is removed and the lymphocytes are fused with cells of the myeloma cell line X63-Ag8.653.3 of Example 1 (B). The fusion is made by means of polyethylene glycol (cf. Goding, J.W., Cell Biology, Biochemistry and Immunology, 3rd edition (1996), Verlag Academic Press Limited, 24-28). Hybridoma cells are obtained. They are incubated in HAT medium at 37°C for 10 to 12 days. The hybridoma cell library 2A is obtained.

Hexapeptides with N-terminal biotin are synthesized. The peptides correspond to the 6C-terminal amino acids of 101 or 118 gene products of *Helicobacter pylory* or *Mycobacter*

tuberculosis. 10^3 cells of the hybridoma cell library 2A are also washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide + 10 µg/ml of the above biotin-labeled peptides. The cells are washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with 10 µg/ml streptavidine FITC. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1 µg/ml propidium iodide and subjected to FACS analysis after excitation with blue light.

It shows that the hybridoma cells have a green fluorescence. This fluorescence is due to the expression of antibodies on the cell surface of the hybridoma cells. Further studies show that the antibodies have an anti-*Helicobacter pylori* or *Mycobacter tuberculosis* activity.

(B)

Cells of the hybridoma cell line U98/6 which produce a mouse anti-urokinase antibody are used. These cells (10^7) are transfected with 20-40 µg of the pSEX11G2* expression vector according to the invention (cf. figure 2). Electroporation is carried out as a transfection technique, which comprises two pulses of 2 ms at 400 V. The cells are incubated for 48 h in incomplete AIM V-medium at 37°C and 5-7.5 % Co_2. Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and subjected to FACS analysis after excitation with blue light.

It shows that the transfected hybridoma cells have a green fluorescence. This fluorescence is due to the expression of

antibodies on the cell surface of the hybridoma cells. Further studies show that the antibodies have anti-urokinase activity.

The resulting hybridoma cells are subjected to G418 selection for 14 to 24 days before they are again incubated with urokinase-biotin and streptaividine-FICS and subjected to FACS analysis as described above. Hybridoma cells which have a strong green fluorescence are subjected to further G418 selection rounds.

The hybridoma cell line U98/6.3.3 is obtained. It stably expresses antibodies on its cell surface.

Example 3: Selection of monoclonal antibodies which are expressed on the cell surface of hybridoma cells by means of an antibody binding protein

10^3 cells of the hybridoma cell line U98/6.3.3 of Example 2 (B) are mixed with 10^7 cells of the hybridoma cell line DOB.L1.3. The latter hybridoma cell line produces an antibody recognizing the C terminus of the human HLA-DO-ß chain. It is expressed on the cell surface by means of an antibody binding protein the same as that in the hybridoma cell line U98/6.3.3 of Example 2(B). The cell mixture is washed with cold DPBS + 0.1 T Na azide and incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cell mixture is incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and supplied to a FACS sorter following excitation with blue light.

Hybridoma cells with green fluorescence are selected. In further studies, they show an anti-urokinase activity. The hybridoma cell lines U98/6.3.3 S1-S50 are obtained.

Example 4: Production and purification of an antibody binding protein according to the invention

(A)

The DNA of figure 1 between nucleotide numbers 682-1782 is provided with BAMHI linkers, subsequently cleaved using BamHI, and inserted in the pQE-8 expression vector cleaved by BamHI (Qiagen company). The expression plasmid pQE-8/antibody binding protein is obtained. Such a plasmid codes for a fusion protein comprising 6 histidine residues (N terminus partner) and the antibody binding protein of fig. 1 according to the invention (C terminus partner). pQE-8/antibody binding protein is used for transforming *E. coli* SG 13009 (cf. Gottesman, S. *et al.*, J. Bacteriol. 148, (1981), 265-273). The bacteria are cultured in an LB broth with 100 µg/ml ampicillin and 25 µg/ml kanamycin and induced with 60 µM isopropyl-ß-D-thiogalactopyranoside (IPTG) for 4 h. Lysis of the bacteria is achieved by the addition of 6 M guanidine hydrochloride. Thereafter, chromatography (Ni-NTA resin) is carried out with the lysate in the presence of 8 M urea in accordance with the instructions from the manufacturer of the chromatography material (Qiagen company). The bound fusion protein is eluted in a buffer having a pH of 3.5. After its neutralization, the fusion protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained with coomassie blue (cf. Thomas, J.O., and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).

It shows that an antibody binding protein (fusion protein) according to the invention can be prepared in highly pure form.

(B)

10^8 cells of the myeloma cell line X63-Ag8.653.3 obtained in Example 1 (B) are washed with PBS, taken up in PBS + 1 % Tween 20 and incubated on ice. Particulate cell components are separated by centrifugation at 30,000 g, and the supernatant is placed on an IgG sepharose column (IgG sepharose 6 Fast Flow Lab Pack from Pharmacia company). Unbound components are removed by washing and the antibody binding protein according to the invention is eluted in acidic pH.

Following its neutralization, the antibody binding protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained using coomassie blue (see above).

It showed that an antibody binding protein (fusion protein) according to the invention can be obtained in highly pure form.

Example 5: Preparation and detection of an antibody according to the invention

A fusion protein of Example 4 according to the invention is subjected to 18 % SDS polyacrylamide gel electrophoresis. After staining the gel with 4 M sodium acetate, an about 41 kD band was excised from the gel and incubated in phosphate-buffered common salt solution. Gel pieces are sedimented before the protein concentration of the supernatant is determined by SDS polyacrylamide gel electrophoresis which

is followed by coomassie blue staining. Animals are immunized with the gel-purified fusion protein as follows:

Immunization protocol for polyclonal antibodies in rabbits

35 µg of gel-purified fusion protein in 0.7 ml PBS and 0.7 ml of complete or incomplete Freund's adjuvant were used per immunization:

Day 0: 1st immunization (complete Freund's adjuvant)

Day 14: 2nd immunization (incomplete Freund's adjuvant; icFA)

Day 28: 3rd immunization (icFA)

Day 56: 4th immunization (icFA)

Day 80: bleeding to death.

The rabbit serum is tested in an immunoblot. For this purpose, a fusion protein of Example 4 according to the invention is subjected to SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (cf. Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10 (1984), 203-209). The Western blot analysis was carried out as described in Bock, C.-T. *et al.*, Virus Genes 8, (1994), 215-229. For this purpose, the nitrocellulose filter is incubated with a first antibody at 37°C for one hour. This antibody is the rabbit serum (1:10000 in PBS). After several wash steps using PBS, the nitrocellulose filter is incubated with a second antibody. This antibody is an alkaline phosphatase-coupled monoclonal goat anti-rabbit IgG antibody (Dianova company) (1:5000) in PBS. 30 minutes of incubation at 37°C are followed by several wash steps using PBS and subsequently by the alkaline phosphatase detection reaction with developer solution (36 µM 5'-bromo-4-chloro-3-indolylphosphate, 400 µM nitro blue tetrazolium, 100 mM

Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) at room temperature until bands are visible.

It shows that polyclonal antibodies according to the invention can be prepared.

Immunization protocol for polyclonal antibodies in chickens

40 µg of gel-purified fusion protein in 0.8 ml PBS and 0.8 ml of complete or incomplete Freund's adjuvant were used per immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
Day 50: 3rd immunization (icFA)

Antibodies are extracted from egg yolk and tested in a Western blot. Polyclonal antibodies according to the invention are detected.

Immunization protocol for monoclonal antibodies in mice

12 µg of gel-purified fusion protein in 0.25 ml PBS and 0.25 ml of complete or incomplete Freund's adjuvant are used per immunization. The fusion protein is dissolved in 0.5 ml (without adjuvant) in the 4th immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
Day 56: 3rd immunization (icFA)
Day 84: 4th immunization (PBS)
Day 87: fusion.

Supernatants of hybridomas are tested in a Western blot. Monoclonal antibodies according to the invention are detected.

Amended Claims

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.

2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.

3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.

4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.

5. The method according to claim 3 or 4, wherein the Fc binding protein is CD16, CD32 or CD64.

6. The method according to any of claims 2 to 5, wherein the antibody binding protein comprises an antibody binding domain of proteins A, G, L or LG.

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig cappa chain or a mouse MHC-class I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.

8. The method according to claim 7, wherein the antibody binding protein is that of figure 1, figure 2 or figure 3.

9. The method according to any of claims 1 to 8, wherein the hybridoma cells (over)express Rag1 and/or Rag2.

10. The method according to any of claims 1 to 9, wherein the antigens originate from an antigen library.

11. The method according to any of claims 1 to 10, wherein the antigens are bound to a carrier.

12. The method according to claim 11, wherein the carrier comprises magnetobeads.

13. The method according to any of claims 1 to 10, wherein the antigens comprise a fluorescence or biotin labeling.

14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.

15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig cappa chain or a mouse MHC-class

I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises the amino acid sequence of figure 1, figure 2 or figure 3 or an amino acid sequence differing therefrom by one or more amino acids.

17. DNA coding for the antibody binding protein according to claim 16, comprising:
    (a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or
    (b) a DNA related to the DNA of (a) via the degenerated code.

18. An expression vector, coding for the DNA according to claim 17.

19. Cells containing the expression vector according to claim 18.

20. An antibody directed against the antibody binding protein according to claim 16.

Abstract of the Disclosure

The present invention relates to a method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, wherein the antibodies are presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and to the binding of the antibodies to antigens. The invention also concerns means usable for this purpose.

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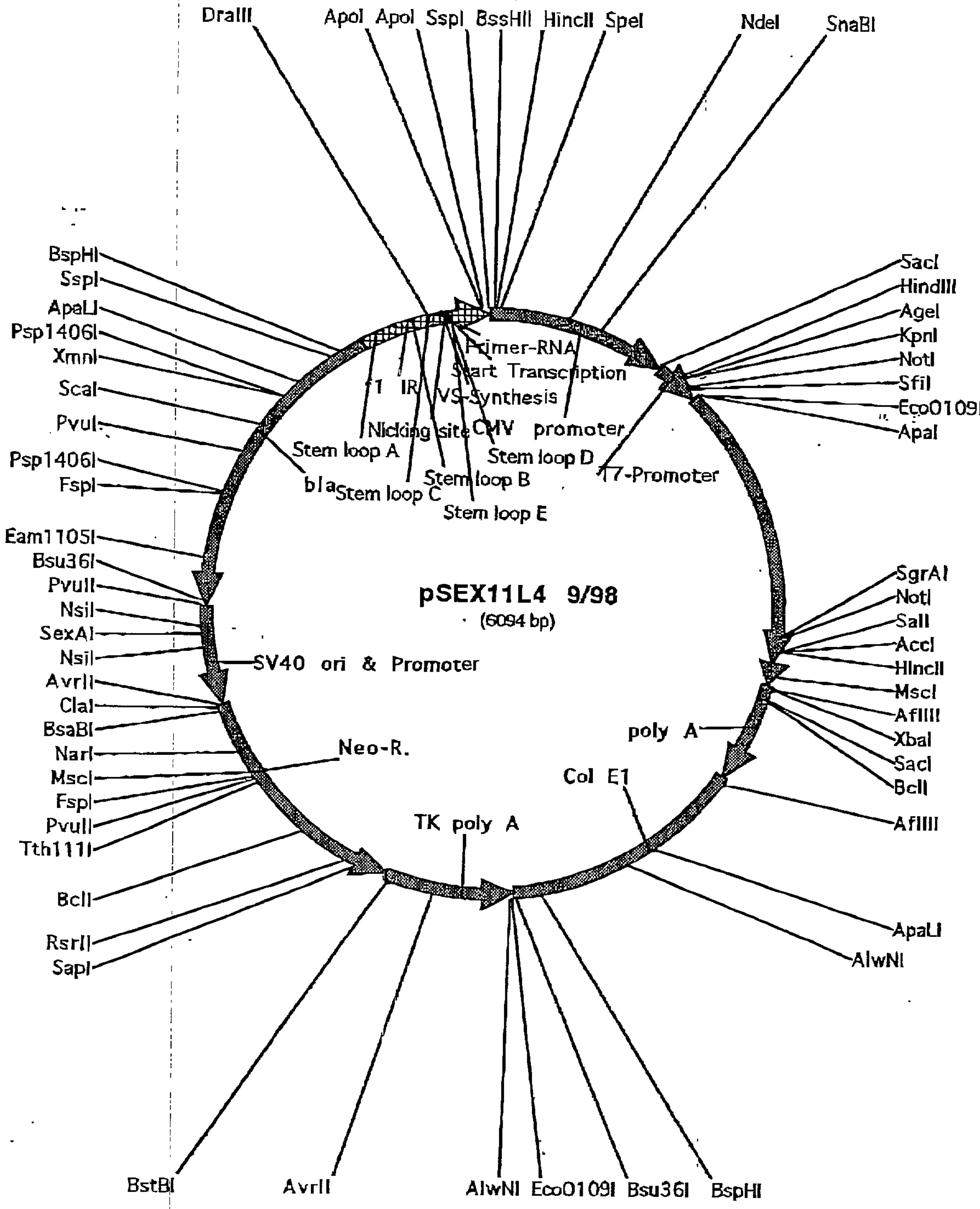

Fig. 1

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    BssHII  HincII           SpeI
   1 GCGCGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA
  60 GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG
 119 CTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
 178 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCAC
                             NdeI
 237 TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG
       CMV   promotor
 296 TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGC
           SnaBI
 355 AGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC
 414 AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGT
 473 CAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACT
                                                            SacI
 532 CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGA
                                                        T7-Promoter
 591 GCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCA
                               AgeI
                        HindIII  KpnI
 650 CTATAGGGAGACCCAAGCTTGGTACCGGTGCGATGGCACCCTGCATGCTGCTCCTGCTG
                                       1▶MetAlaProCysMetLeuLeuLeuLeu
        SfiI                               ApaI
       NotI                              Eco0109I
 709 TTGGCGGCCGCCCTGGCCCCGACTCAGACCCGCGCGGGGGCCCAAAAGGAGAAGACCCC
  10▶LeuAlaAlaAlaLeuAlaProThrGlnThrArgAlaGlyAlaGlnLysGluLysThrPr
 768 CGAGGAGCCCAAGGAGGAGGTGACCATCAAGGCCAACCTGATCTACGCCGACGGCAAGA
  29▶oGluGluProLysGluGluValThrIleLysAlaAsnLeuIleTyrAlaAspGlyLysT
 827 CCCAGACCGCCGAGTTCAAGGGCACCTTCGAGGAGGCCACCGCGGAGGCCTACCGCTAC
  49▶hrGlnThrAlaGluPheLysGlyThrPheGluGluAlaThrAlaGluAlaTyrArgTyr
 886 GCCGACGCCCTGAAGAAGGACAACGGCGAGTACACCGTGGACGTGGCCGACAAGGGCTA
  69▶AlaAspAlaLeuLysLysAspAsnGlyGluTyrThrValAspValAlaAspLysGlyTy
 945 CACCCTGAACATCAAGTTCGCCGGCAAGGAGAAGACCCCCGAGGAGCCCAAGGAGGAGG
  88▶rThrLeuAsnIleLysPheAlaGlyLysGluLysThrProGluGluProLysGluGluV
1004 TGACCATCAAGGCCAACCTGATCTACGCCGACGGCAAGACCCAGACCGCCGAGTTCAAG
 108▶alThrIleLysAlaAsnLeuIleTyrAlaAspGlyLysThrGlnThrAlaGluPheLys
1063 GGCACCTTCGAGGAGGCCACCGCGGAGGCCTACCGCTACGCCGACGCCCTGAAGAAGGA
 128▶GlyThrPheGluGluAlaThrAlaGluAlaTyrArgTyrAlaAspAlaLeuLysLysAs
1122 CAACGGCGAGTACACCGTGGACGTGGCCGACAAGGGCTACACCCTGAACATCAAGTTCG
 147▶pAsnGlyGluTyrThrValAspValAlaAspLysGlyTyrThrLeuAsnIleLysPheA
1181 CCGGCAAGGAGAAGACCCCCGAGGAGCCCAAGGAGGAGGTGACCATCAAGGCCAACCTG
 167▶laGlyLysGluLysThrProGluGluProLysGluGluValThrIleLysAlaAsnLeu
1240 ATCTACGCCGACGGCAAGACCCAGACCGCCGAGTTCAAGGGCACCTTCGAGGAGGCCAC
 187▶IleTyrAlaAspGlyLysThrGlnThrAlaGluPheLysGlyThrPheGluGluAlaTh
1299 CGCGGAGGCCTACCGCTACGCCGACGCCCTGAAGAAGGACAACGGCGAGTACACCGTGG
 206▶rAlaGluAlaTyrArgTyrAlaAspAlaLeuLysLysAspAsnGlyGluTyrThrValA
1358 ACGTGGCCGACAAGGGCTACACCCTGAACATCAAGTTCGCCGGCAAGGAGAAGACCCCC
 226▶spValAlaAspLysGlyTyrThrLeuAsnIleLysPheAlaGlyLysGluLysThrPro
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Fig. 1 (cont'd I)

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1417 GAGGAGCCCAAGGAGGAGGTGACCATCAAGGCCAACCTGATCTACGCCGACGGCAAGAC
 246▶GluGluProLysGluGluValThrIleLysAlaAsnLeuIleTyrAlaAspGlyLysTh
1476 CCAGACCGCCGAGTTCAAGGGCACCTTCGAGGAGGCCACCGCGGAGGCCTACCGCTACG
 265▶rGlnThrAlaGluPheLysGlyThrPheGluGluAlaThrAlaGluAlaTyrArgTyrA
1535 CCGACGCCCTGAAGAAGGACAACGGCGAGTACACCGTGGACGTGGCCGACAAGGGCTAC
 285▶laAspAlaLeuLysLysAspAsnGlyGluTyrThrValAspValAlaAspLysGlyTyr
                   SgrAI   NotI
1594 ACCCTGAACATCAAGTTCGCCGGCGCGGCCGCAGAACAAAAACTCATCTCAGAAGAGGA
 305▶ThrLeuAsnIleLysPheAlaGlyAlaAlaAlaGluGlnLysLeuIleSerGluGluAs

               SalI
               HincII
               AccI
1653 TCTGAATGGGGCCGTCGACGGACAAAACGACACCAGCCAAACCAGCAGCCCCTCAGCAT
 324▶pLeuAsnGlyAlaValAspGlyGlnAsnAspThrSerGlnThrSerSerProSerAlaS

                                         MscI
1712 CCAGCAACATAAGCGGAGGCATTTTCCTTTTCTTCGTGGCCAATGCCATAATCCACCTC
 344▶erSerAsnIleSerGlyGlyIlePheLeuPhePheValAlaAsnAlaIleIleHisLeu

                    AflIII XbaI                             SacI
1771 TTCTGCTTCAGTTGAGGTGACACGTCTAGAGCTATTCTATAGTGTCACCTAAATGCTAG
 364▶PheCysPheSer•••

        BclI
1830 AGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCT

                                                 poly A
1889 CCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAAT

1948 GAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGG

2007 GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGG

2066 GCTCTATGGCTTCTGAGGCGGAAAGAACCAGTGGCGGTAATACGGTTATCCACAGAATC
                     AflIII
2125 AGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA
2184 AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAA

2243 AATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTT

2302 TCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACC

2361 TGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTAT

                                             ApaLI
2420 CTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCA

     Col E1
2479 GCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACG

                   AlwNI
2538 ACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGC

2597 GGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATT

2656 TGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGAT

2715 CCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACG

2774 CGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCA
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Fig. 1 (cont'd II)

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                                            BspHI
2833 GTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCA
2892 CCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAA
                     Eco0109I
     Bsu36I        AlwNI
2951 CCTGAGGCTATGGCAGGGCCTGCCGCCCCGACGTTGGCTGCGAGCCCTGGGCCTTCACC
3010 CGAACTTGGGGGGTGGGGTGGGGAAAAGGAAGAAACGCGGGCGTATTGGCCCCAATGGG
3069 GTCTCGGTGGGGTATCGACAGAGTGCCAGCCCTGGGACCGAACCCCGCGTTTATGAACA
      TK poly A
3128 AACGACCCAACACCGTGCGTTTTATTCTGTCTTTTTATTGCCGTCATAGCGCGGGTTCC
                                                Avrll
3187 TTCCGGTATTGTCTCCTTCCGTGTTTCAGTTAGCCTCCCCCTAGGGTGGGCGAAGAACT
3246 CCAGCATGAGATCCCCGCGCTGGAGGATCATCCAGCCGGCGTCCCGGAAAACGATTCCG
3305 AAGCCCAACCTTTCATAGAAGGCGGCGGTGGAATCGAAATCTCGTGATGGCAGGTTGGG
                     BstBI
3364 CGTCGCTTGGTCGGTCATTTCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAG
                                        263◄•••PhePheGluAspLeuLeu
3423 GCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGC
 256◄ArgTyrPheAlaIleArgGlnSerAspProAlaAlaIleGlyTyrLeuValLeuPheAr
                           SapI
3482 GGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCC
 236◄gAspAlaTrpGluGlyGlyLeuGluGluAlaIleAspArgThrAlaLeuAlaIleAspG
          .RsrII
3541 TGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATT
 216◄lnTyrArgAspAlaValGlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsn
3600 TTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGT
 197◄GluValMetIleAsnProLeuCysAlaAspGlyHisThrValValLeuAspGluGlyAs
3659 CGGGCATGCTCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCT
 177◄pProMetSerAlaLysLeuArgAlaPheLeuGluAlaProAlaLeuGlyGlnHisGluG
     BclI
3718 TGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATG
 157◄lnAspAspGlnAspValLeuGlyAlaGluMetArgThrArgAlaArgGluIleArgHis
3777 TTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTG
 138◄LysAlaGlnHisAspPheProCysThrAlaProAspLeuThrHisLeuArgArgMetAl
3836 CATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGC
 118◄aAspAlaMetIleSerValLysGluAlaProAlaLeuHisSerSerLeuLeuAspGlnG
                                                 Tth111I     PvuII
3895 CCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCAC
  98◄lyProValGluGlyLeuLeuLeuTrpAspArgGlyAlaGluThrValValAspLeuVal
                                  Neo-R.
      FspI                 MscI
3954 AGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCTTGCA
  79◄AlaAlaCysProValGlyThrThrAlaLeuTrpSerLeuArgAlaAlaGluAspGlnLe
                                                    NarI
4013 GTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCT
  59◄uGluAsnLeuAlaGlySerLeuAspThrLysValPheLeuValProArgGlyGlnAlaS
4072 GACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCC
  39◄erLeuArgPheValAlaAlaAspSerCysGlyIleThrGlnGlnAlaTrpAspTyrGly
4131 GAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATCA
  20◄PheLeuArgGluValTrpAlaAlaProSerGlyAlaHisLeuGlyAspGlnGluIleMe
           BsaBI              ClaI               AvrII
4190 TGCGAAACGATCCTCATCCTGTCTCTTGATCGATCTTTGCAAAAGCCTAGGCCTCCAAA
   0◄t
4249 AAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGAGGCGGCCTCGGCCTCTG
4308 CATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTT
```

Fig. 1 (cont'd III)

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                      SV40 ori & Promoter                     NsiI
4367 AGGGGCGGGATGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCAT

                                                   SexAI
4426 GCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACT

          NsiI
4485 AATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACAC

                        PvuII            Bsu36I
4544 CCTAACTGACACACATTCCACAGCTGGTTCTTTCCGCCTCAGGACTCTTCCTTTTTCAA

4603 TAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCA
                                                287◄•••TrpHisLysIleLe
                                                            Eam1105I
4662 GTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCC
 281◄uSerAlaGlyIleGluAlaIleGlnArgAsnArgGluAspMetThrAlaGlnSerGlyT
4721 GTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
 261◄hrThrTyrIleValValIleArgSerProLysGlyAspProGlyLeuAlaAlaIleIle
4780 ACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAA
 242◄GlyArgSerGlyArgGluGlyAlaGlySerLysAspAlaIlePheTrpGlyAlaProLe
4839 GGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGT
 222◄uAlaSerArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnG
                                                 FspI  Psp1406I
4898 TGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCAT
 202◄lnArgSerAlaLeuThrLeuLeuGluGlyThrLeuLeuLysArgLeuThrThrAlaMet
4957 TGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTT
 183◄AlaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeuGluProGl
5016 CCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
 163◄uTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLeuGluL
                 PvuI
5075 TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTAT
 143◄ysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSerMetThrIle
                                                           bla
5134 GGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTG
 124◄AlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLysGluThrValPr
       ScaI
5193 GTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGC
 104◄oSerTyrGluValLeuAspAsnGlnSerTyrHisIleArgArgGlyLeuGlnGluGlnG
5252 CCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCAT
  84◄lyAlaAspIleArgSerLeuValAlaGlyCysLeuLeuValLysPheThrSerMetMet
        Psp1406I
      XmnI
5311 TGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT
  65◄ProPheArgGluGluProArgPheSerGluLeuIleLysGlySerAsnLeuAspLeuGl
                ApaLI
5370 CGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTT
  45◄uIleTyrGlyValArgAlaGlyLeuGlnAspGluAlaAspLysValLysValLeuThrG
5429 TCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACG
  25◄luProHisAlaPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArg
                                          SspI
5488 GAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTT
   6◄PheHisGlnIleSerMet
        BspHI
5547 ATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTT
5606 CCGCGCACATTTCCCCGAAAAGTGCCACCTGACGCGCCCTGTAGCGGCGCATTAAGCGC

                                          Stem loop A
5665 GGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCG
```

Fig. (cont'd IV)

5724 CTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCT

f1 IR Stem loop B

5783 CTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAA

DraIII Stem loop C Primer-RNA

5842 AAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTC

Start Transcription

VS-Synthese Nicking site Stem loop D Stem loop E

5901 GCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACA

5960 ACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGC

ApoI ApoI SspI

6019 CTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATAT

6078 TAACGCTTACAATTTAC

Fig. 1 (cont'd V)

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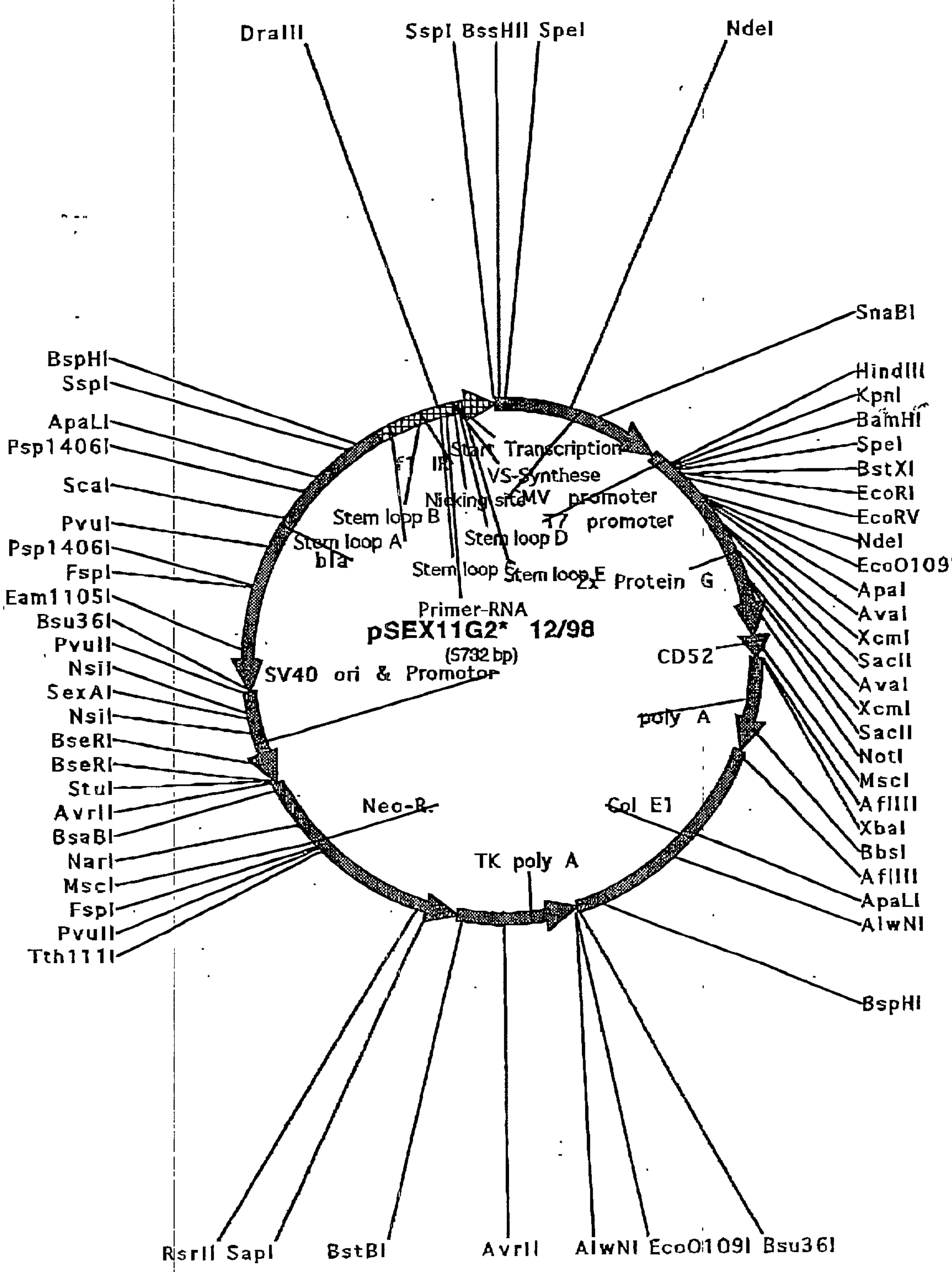

Fig. 2

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BssHII Spel
1 GCGCGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGT
55 CATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATG
109 GCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGT
163 ATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
Ndel
217 ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTA
CMV promoter
271 CGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
SnaBI
325 ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCG
379 CTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGT
433 TTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGT
487 TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCAT
541 TGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTC
T7 promoter
595 TCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTC
HindIIIKpnI BamHI SpeI BstXI
649 ACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGC
EcoRI EcoRV
703 CAGTGTGCTGGAATTCGGCTTGGGGATATCCACCATGGAGACAGACACACTCCT
1▸ MetGluThrAspThrLeuLe
NdeI
757 GCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACTATCCATATGA
7▸ uLeuTrpValLeuLeuLeuTrpValProGlySerThrGlyAspTyrProTyrAs
ApaI
Eco0109I AvaI
811 TGTTCCAGATTATGCTGGGGCCCAAAAGCCCGAGGTGATCGATGCCAGCGAGCT
25▸ pValProAspTyrAlaGlyAlaGlnLysProGluValIleAspAlaSerGluLe
865 GACCCCCGCCGTGACCACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGG
43▸ uThrProAlaValThrThrTyrLysLeuValIleAsnGlyLysThrLeuLysGl
XcmI SacII
919 CGAGACCACCACCGAGGCCGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACA
61▸ yGluThrThrThrGluAlaValAspAlaAlaThrAlaGluLysValPheLysGl
973 ATACGCTAATGACAACGGGGTCGACGGCGAGTGGACTTACGACGACGCCACCAA
79▸ nTyrAlaAsnAspAsnGlyValAspGlyGluTrpThrTyrAspAspAlaThrLy
AvaI
2x Protein G
1027 GACCTTCACCGTGACCGAGAAGCCCGAGGTGATCGATGCCAGCGAGCTGACCCC
97▸ sThrPheThrValThrGluLysProGluValIleAspAlaSerGluLeuThrPr

Fig. 2 (cont'd I)

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```
1081 CGCCGTGACCACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAGAC
 115▸oAlaValThrThrTyrLysLeuValIleAsnGlyLysThrLeuLysGlyGluTh

      XcmI                          SacII
1135 CACCACCGAGGCCGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACAATACGC
 133▸rThrThrGluAlaValAspAlaAlaThrAlaGluLysValPheLysGlnTyrAl
1189 TAATGACAACGGGGTCGACGGCGAGTGGACTTACGACGACGCCACCAAGACCTT
 151▸aAsnAspAsnGlyValAspGlyGluTrpThrTyrAspAspAlaThrLysThrPh
                    NotI
1243 CACCGTGACCGAGGCGGCCGCAGAACAAAAACTCATCTCAGAAGAGGATCTGAA
 169▸eThrValThrGluAlaAlaAlaGluGlnLysLeuIleSerGluGluAspLeuAs

1297 TGGGGCCGTCGACGGACAAAACGACACCAGCCAAACCAGCAGCCCCTCAGCATC
 187▸nGlyAlaValAspGlyGlnAsnAspThrSerGlnThrSerSerProSerAlaSe

              CD52                          MscI
1351 CAGCAACATAAGCGGAGGCATTTTCCTTTTCTTCGTGGCCAATGCCATAATCCA
 205▸rSerAsnIleSerGlyGlyIlePheLeuPhePheValAlaAsnAlaIleIleHi

                                 AflIIIXbaI
1405 CCTCTTCTGCTTCAGTTGAGGTGACACGTCTAGAGCTATTCTATAGTGTCACCT
 223▸sLeuPheCysPheSer***

1459 AAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC

1513 TGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC

     poly A
1567 TGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCA

                                                   BbsI
1621 TTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGA

1675 CAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAG

1729 AACCAGTGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGA
     AflIII
1783 ACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGC

1837 TGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCT

1891 CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCC

1945 CTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACC

1999 TGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA

                                                 ApaLI
2053 GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAAC

             Col E1
2107 CCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA

                                        AlwNI
2161 ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTA

2215 GCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACT

2269 ACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA

2323 CCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTA
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Fig. 2 (cont'd II)

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2377 GCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTC

2431 AAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACT
                                     BspHI
2485 CACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCC
                                                        Bsu36I
2539 TTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACCTG
               EcoO109I
             AlwNI
2593 AGGCTATGGCAGGGCCTGCCGCCCCGACGTTGGCTGCGAGCCCTGGGCCTTCAC

2647 CCGAACTTGGGGGGTGGGGTGGGGAAAAGGAAGAAACGCGGGCGTATTGGCCCC

2701 AATGGGGTCTCGGTGGGGTATCGACAGAGTGCCAGCCCTGGGACCGAACCCCGC

                TK poly A
2755 GTTTATGAACAAACGACCCAACACCGTGCGTTTTATTCTGTCTTTTTATTGCCG

2809 TCATAGCGCGGGTTCCTTCCGGTATTGTCTCCTTCCGTGTTTCAGTTAGCCTCC

  AvrII
2863 CCCTAGGGTGGGCGAAGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATCC

2917 AGCCGGCGTCCCGGAAAACGATTCCGAAGCCCAACCTTTCATAGAAGGCGGCGG
                                                       BstBI
2971 TGGAATCGAAATCTCGTGATGGCAGGTTGGGCGTCGCTTGGTCGGTCATTTCGA
3025 ACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCG
               263◄ •••PhePheGluAspLeuLeuArgTyrPheAlaIleArg
3079 CTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTC
 250◄GlnSerAspProAlaAlaIleGlyTyrLeuValLeuPheArgAspAlaTrpGlu
          SapI                                         RsrII
3133 GCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCG
 232◄GlyGlyLeuGluGluAlaIleAspArgThrAlaLeuAlaIleAspGlnTyrArg
3187 GTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTC
 214◄AspAlaValGlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsnGlu
3241 CACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCC
 196◄ValMetIleAsnProLeuCysAlaAspGlyHisThrValValLeuAspGluGly
3295 GTCGGGCATGCTCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTG
 178◄AspProMetSerAlaLysLeuArgAlaPheLeuGluAlaProAlaLeuGlyGln
3349 ATGCTCTTGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCG
 160◄HisGluGlnAspAspGlnAspValLeuGlyAlaGluMetArgThrArgAlaArg
3403 CTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGT
 142◄GluIleArgHisLysAlaGlnHisAspPheProCysThrAlaProAspLeuThr
3457 ATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAG
 124◄HisLeuArgArgMetAlaAspAlaMetIleSerValLysGluAlaProAlaLeu
3511 GTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCT
 106◄HisSerSerLeuLeuAspGlnGlyProValGluGlyLeuLeuLeuTrpAspArg
                                    FspI                Neo-R.
               Tth111I      PvuII                      MscI
3565 TCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGC
  88◄GlyAlaGluThrValValAspLeuValAlaAlaCysProValGlyThrThrAla
3619 CAGCCACGATAGCCGCGCTGCCTCGTCTTGCAGTTCATTCAGGGCACCGGACAG
  70◄LeuTrpSerLeuArgAlaAlaGluAspGlnLeuGluAsnLeuAlaGlySerLeu
                                NarI
3673 GTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGC
  52◄AspThrLysValPheLeuValProArgGlyGlnAlaSerLeuArgPheValAla
3727 GGCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTC
  34◄AlaAspSerCysGlyIleThrGlnGlnAlaTrpAspTyrGlyPheLeuArgGlu
3781 CACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATCATGCGAAA
  16◄ValTrpAlaAlaProSerGlyAlaHisLeuGlyAspGlnGluIleMet
                                                  StuI
   BsaBI                                        AvrII
3835 CGATCCTCATCCTGTCTCTTGATCGATCTTTGCAAAAGCCTAGGCCTCCAAAAA
```

Fig. 2 (cont'd III)

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      BseRI                                  BseRI
3889 AGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGAGGCGGCCTCGGCCT

3943 CTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGG

                                  SV40 ori & Promotor
3997 GCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACT

          NsiI
4051 AATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTC

      SexAI                    NsiI
4105 CACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTG

                                                   PvuII
4159 GGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACAGCTGGTTCT

        Bsu36I
4213 TTCCGCCTCAGGACTCTTCCTTTTTCAATAAATCAATCTAAAGTATATATGAGT
4267 AAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGA
                   287◄•••TrpHisLysIleLeuSerAlaGlyIleGluAlaIl
                                         Eam1105I
4321 TCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTA
 274◄eGlnArgAsnArgGluAspMetThrAlaGlnSerGlyThrThrTyrIleValVa
4375 CGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACC
 256◄lIleArgSerProLysGlyAspProGlyLeuAlaAlaIleIleGlyArgSerGl
4429 CACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCG
 238◄yArgGluGlyAlaGlySerLysAspAlaIlePheTrpGlyAlaProLeuAlaSe
4483 AGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTT
 220◄rArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnGl
                                                FspI Psp1406I
4537 GCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTG
 202◄nArgSerAlaLeuThrLeuLeuGluGlyThrLeuLeuLysArgLeuThrThrAl
4591 CCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCA
 184◄aMetAlaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLe
4645 GCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAA
 166◄uGluProGluTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPh
                                  PvuI
4699 AAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAG
 148◄eAlaThrLeuGluLysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaTh
4753 TGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT
 130◄rAsnAspSerMetThrIleAlaAlaSerCysLeuGluArgValThrMetGlyAs
                bla          ScaI
4807 CCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAAT
 112◄pThrLeuHisLysGluThrValProSerTyrGluValLeuAspAsnGlnSerTy
4861 AGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCG
  94◄rHisIleArgArgGlyLeuGlnGluGlnGlyAlaAspIleArgSerLeuValAl
                                                Psp1406I
4915 CGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGC
  76◄aGlyCysLeuLeuValLysPheThrSerMetMetProPheArgGluGluProAr
4969 GAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTC
  58◄gPheSerGluLeuIleLysGlySerAsnLeuAspLeuGluIleTyrGlyValAr
     ApaLI
5023 GTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAG
  40◄gAlaGlyLeuGlnAspGluAlaAspLysValLysValLeuThrGluProHisAl
5077 CAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAAT
  22◄aPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArgPheHi
                              SspI
5131 GTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTT
   4◄sGlnIleSerMet
        BspHI
5185 ATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAG
```

Fig. 2 (cont'd IV)

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5239 GGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGCGCCCTGTAGCGGCG

Stem loop A

5293 CATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCA

5347 GCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCG

f1 IR Stem loop B

5401 CCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTA

DraIII

5455 GTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTA

Stem loop C Primer-RNA Start Transcription VS-Synthesis

5509 GTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGT

Nicking site Stem loop D Stem loop E

5563 TCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGG

5617 TCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAA

SspI

5671 ATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTA

5725 CAATTTAC

Fig. 2 (cont'd V)

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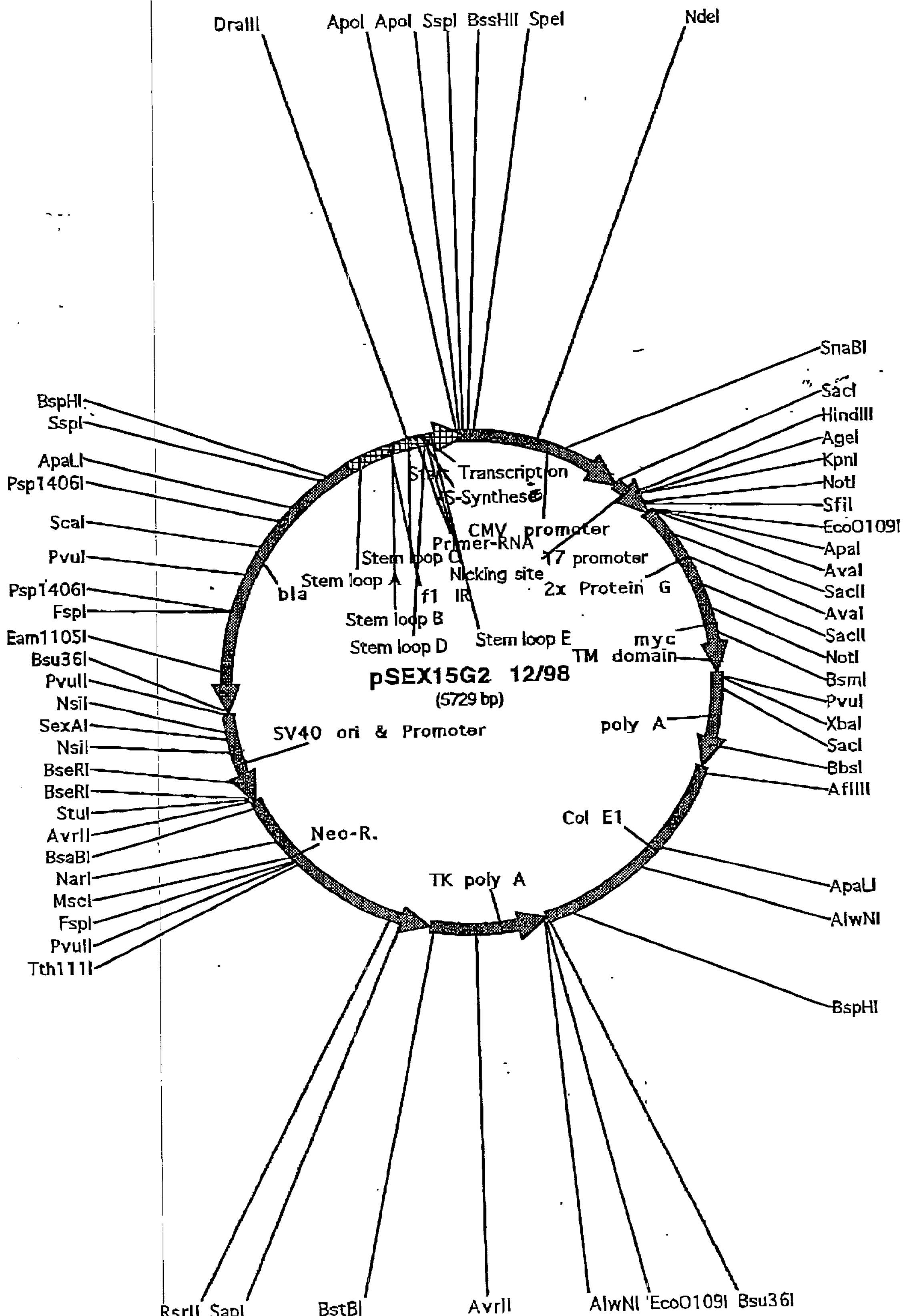

Fig. 3

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     BssHII                   SpeI
   1 GCGCGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCA

  57 TTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCC

 113 GCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTC

 169 CCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGG

                                                 NdeI
 225 TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTAT

                          CMV  promoter
 281 TGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTAT

                                   SnaBI
 337 GGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTG

 393 ATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATT

 449 TCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAAC

 505 GGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGG

                                    SacI
 561 CGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCAC

                                T7 promoter          HindIII  KpnI
 617 TGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTGGT

                                                 SfiI
     AgeI                                      NotI
 673 ACCGGTGCGATGGCACCCTGCATGCTGCTCCTGCTGTTGGCGGCCGCCCTGGCCCC
                1▶MetAlaProCysMetLeuLeuLeuLeuLeuAlaAlaAlaLeuAlaPr
                     ApaI
                  Eco0109I     AvaI
 729 GACTCAGACCCGCGCGGGGGCCCAAAAGCCCGAGGTGATCGATGCCAGCGAGCTGA
  16▶oThrGlnThrArgAlaGlyAlaGlnLysProGluValIleAspAlaSerGluLeuT
 785 CCCCCGCCGTGACCACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAG
  35▶hrProAlaValThrThrTyrLysLeuValIleAsnGlyLysThrLeuLysGlyGlu

                                    SacII
 841 ACCACCACCGAGGCCGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACAATACGC
  54▶ThrThrThrGluAlaValAspAlaAlaThrAlaGluLysValPheLysGlnTyrAl
 897 TAATGACAACGGGGTCGACGGCGAGTGGACTTACGACGACGCCACCAAGACCTTCA
  72▶aAsnAspAsnGlyValAspGlyGluTrpThrTyrAspAspAlaThrLysThrPheT
                   AvaI
              2x Protein G
 953 CCGTGACCGAGAAGCCCGAGGTGATCGATGCCAGCGAGCTGACCCCCGCCGTGACC
  91▶hrValThrGluLysProGluValIleAspAlaSerGluLeuThrProAlaValThr
1009 ACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAGACCACCACCGAGGC
 110▶ThrTyrLysLeuValIleAsnGlyLysThrLeuLysGlyGluThrThrThrGluAl

                   SacII
1065 CGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACAATACGCTAATGACAACGGGG
 128▶aValAspAlaAlaThrAlaGluLysValPheLysGlnTyrAlaAsnAspAsnGlyV
```

Fig. 3 (cont'd I)

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NotI

1121 TCGACGGCGAGTGGACTTACGACGACGCCACCAAGACCTTCACCGTGACCGAGGCG
147 alAspGlyGluTrpThrTyrAspAspAlaThrLysThrPheThrValThrGluAla

myc

1177 GCCGCAGAACAAAAACTCATCTCAGAAGAGGATCTGAATGGGGCCGTCGACGAACA
166 AlaAlaGluGlnLysLeuIleSerGluGluAspLeuAsnGlyAlaValAspGluGl

BsmI

1233 AAAACTCATCTCAGAAGAGGATCTGAATGCTGTGGGCCAGGACACGCAGGAGGTCA
184 nLysLeuIleSerGluGluAspLeuAsnAlaValGlyGlnAspThrGlnGluVall

1289 TCGTGGTGCCACACTCCTTGCCCTTTAAGGTGGTGGTGATCTCAGCCATCCTGGCC
203 leValValProHisSerLeuProPheLysValValValIleSerAlaIleLeuAla

TM domain

1345 CTGGTGGTGCTCACCATCATCTCCCTTATCATCCTCATCATGCTTTGGCAGAAGAA
222 LeuValValLeuThrIleIleSerLeuIleIleLeuIleMetLeuTrpGlnLysLy

PvuI XbaI

1401 GCCACGTTCGTCGGCCGATCGAGAATCCATCTAGAGCTATTCTATAGTGTCACCTA
240 sProArgSerSerAlaAspArgGluSerIle•••

SacI

1457 AATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGT

poly A

1513 TGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCC

1569 TTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATT

BbsI

1625 CTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAG

1681 GCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGTGGCG

AflIII

1737 GTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAA
1793 AGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATA

1849 GGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA

1905 AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCG

1961 CTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGG

2017 GAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC

ApaLI ColE1

2073 GTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGC

2129 CTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC

AlwNI

2185 TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACA

2241 GAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTAT

2297 CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCG

2353 GCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACG

2409 CGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGC

Fig. 3 (cont'd II)

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                                                    BspHI
2465 TCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGA
2521 TCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATA
                                   EcoO109I
             Bsu36I               AlwNI
2577 TATGAGTAACCTGAGGCTATGGCAGGGCCTGCCGCCCCGACGTTGGCTGCGAGCCC

2633 TGGGCCTTCACCCGAACTTGGGGGGTGGGGTGGGGAAAAGGAAGAAACGCGGGCGT

2689 ATTGGCCCCAATGGGGTCTCGGTGGGGTATCGACAGAGTGCCAGCCCTGGGACCGA

                              TK poly A
2745 ACCCCGCGTTTATGAACAAACGACCCAACACCGTGCGTTTTATTCTGTCTTTTTAT

2801 TGCCGTCATAGCGCGGGTTCCTTCCGGTATTGTCTCCTTCCGTGTTTCAGTTAGCC

       AvrII
2857 TCCCCCTAGGGTGGGCGAAGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATC

2913 CAGCCGGCGTCCCGGAAAACGATTCCGAAGCCCAACCTTTCATAGAAGGCGGCGGT
                                                       BstBI
2969 GGAATCGAAATCTCGTGATGGCAGGTTGGGCGTCGCTTGGTCGGTCATTTCGAACC
3025 CCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCG
                 263◄•••PhePheGluAspLeuLeuArgTyrPheAlaIleArgGlnSe
3081 AATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCA
 248◄rAspProAlaAlaIleGlyTyrLeuValLeuPheArgAspAlaTrpGluGlyGlyL
      SapI                                            RsrII
3137 AGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCAC
 229◄euGluGluAlaIleAspArgThrAlaLeuAlaIleAspGlnTyrArgAspAlaVal
3193 ACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATGATAT
 211◄GlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsnGluValMetIleAs
3249 TCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCTC
 192◄nProLeuCysAlaAspGlyHisThrValValLeuAspGluGlyAspProMetSerA
3305 GCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTGATCATC
 173◄laLysLeuArgAlaPheLeuGluAlaProAlaLeuGlyGlnHisGluGlnAspAsp
3361 CTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCG
 155◄GlnAspValLeuGlyAlaGluMetArgThrArgAlaArgGluIleArgHisLysAl
3417 CTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCA
 136◄aGlnHisAspPheProCysThrAlaProAspLeuThrHisLeuArgArgMetAlaA
3473 TCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTG
 117◄spAlaMetIleSerValLysGluAlaProAlaLeuHisSerSerLeuLeuAspGln
                                                      Tth111I
3529 CCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGA
  99◄GlyProValGluGlyLeuLeuLeuTrpAspArgGlyAlaGluThrValValAspLe
                                      Neo-R.
        PvuIIFspI                  MscI
3585 GCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCG
  80◄uValAlaAlaCysProValGlyThrThrAlaLeuTrpSerLeuArgAlaAlaGluA
                                                         NarI
3641 TCTTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCG
  61◄spGlnLeuGluAsnLeuAlaGlySerLeuAspThrLysValPheLeuValProArg
3697 CCCCTGCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTG
  43◄GlyGlnAlaSerLeuArgPheValAlaAlaAspSerCysGlyIleThrGlnGlnAl
3753 CCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAAT
  24◄aTrpAspTyrGlyPheLeuArgGluValTrpAlaAlaProSerGlyAlaHisLeuG
                                 BsaBI
3809 CCATCTTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATCGATCTTTGC
   5◄lyAspGlnGluIleMet
              StuI
        AvrII                     BseRI
3865 AAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCC
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Fig. 3 (cont'd III)

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      BseRI
3921  GAGGAGGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGG

                                             SV40 ori & Promoter
3977  AGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGA

                               NsiI
4033  CTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAG

                    SexAI                        NsiI
4089  CCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACT

                                                          PvuII
4145  TCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACA

                   Bsu36I
4201  GCTGGTTCTTTCCGCCTCAGGACTCTTCCTTTTTCAATAAATCAATCTAAAGTATA
4257  TATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTC
                               287◄•••TrpHisLysIleLeuSerAlaGlyIleGlu
                                                Eam1105I
4313  AGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAA
 276◄AlaIleGlnArgAsnArgGluAspMetThrAlaGlnSerGlyThrThrTyrIleVa
4369  CTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAC
 257◄lValIleArgSerProLysGlyAspProGlyLeuAlaAlaIleIleGlyArgSerG
4425  CCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGA
 238◄lyArgGluGlyAlaGlySerLysAspAlaIlePheTrpGlyAlaProLeuAlaSer
4481  GCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCC
 220◄ArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnGlnAr
                                                 FspI  Psp1406I
4537  GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATT
 201◄gSerAlaLeuThrLeuLeuGluGlyThrLeuLeuLysArgLeuThrThrAlaMetA
4593  GCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGG
 182◄laValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeuGluPro
4649  TTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTA
 164◄GluTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLe
                   PvuI
4705  GCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTC
 145◄uGluLysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSerM
                                                           bla
4761  ATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTT
 126◄etThrIleAlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLys
                   ScaI
4817  TTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGAC
 108◄GluThrValProSerTyrGluValLeuAspAsnGlnSerTyrHisIleArgArgGl
4873  CGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACT
  89◄yLeuGlnGluGlnGlyAlaAspIleArgSerLeuValAlaGlyCysLeuLeuValL
                            Psp1406I
4929  TTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTT
  70◄ysPheThrSerMetMetProPheArgGluGluProArgPheSerGluLeuIleLys
                                               ApaLI
4985  ACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAG
  52◄GlySerAsnLeuAspLeuGluIleTyrGlyValArgAlaGlyLeuGlnAspGluAl
5041  CATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCC
  33◄aAspLysValLysValLeuThrGluProHisAlaPheValProLeuCysPheAlaA
5097  GCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTT
  14◄laPhePheProIleLeuAlaValArgPheHisGlnIleSerMet
        SspI                                  BspHI
5153  TCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTG
5209  AATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTG
5265  CCACCTGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCG
```

Fig. 3 (cont'd IV)

Stem loop A
5321 CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCC

5377 CTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTC

f1 IR Stem loop B
5433 CCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTA

DraIII Stem loop C Primer-RNA
5489 GGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA

Start Transcription
VS-Synthese Nicking site Stem loop D Stem loop E
5545 CGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTC

5601 AACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTA

ApoI ApoI SspI
5657 TTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATAT

5713 TAACGCTTACAATTTAC

Fig. 3 (cont'd V)